**SUPPORTING INFORMATION**

**Title:**

High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR

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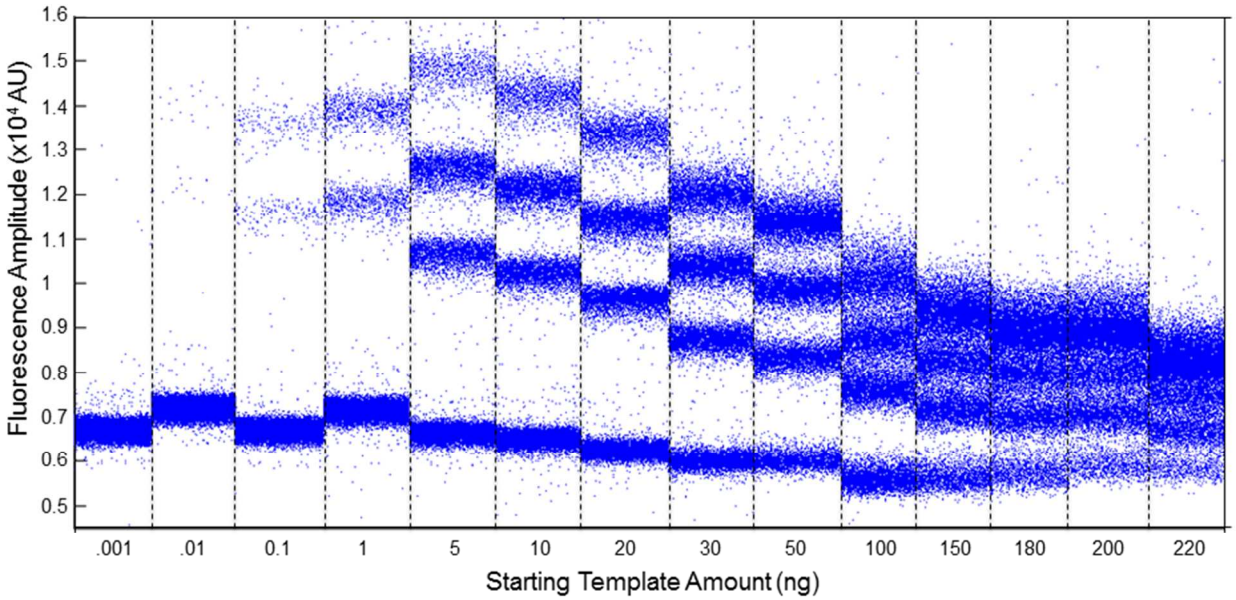


**Figure S-1. Effect of starting template amount on fluorescence amplitude.** Each column is one multiplexed reaction with UC1 and FLT3 primers (~20,000 droplets). Starting template amount refers to the high-quality NA18507 loaded into each 20µl PCR reaction.

**Table S-1. *FLT3* copy number and error with increasing template amounts.**

| Template Amount (ng)* | Copy Number (copies/genome) | StdDev |
|---|---|---|
| 0.001 | - | - |
| 0.01 | 1.33 | 2.6** |
| 0.1 | 3.05 | 0.870 |
| 1 | 2.13 | 0.085 |
| 5 | 2.08 | 0.064 |
| 20 | 2.05 | 0.003 |

*Total ng amount added to each 20ul reaction

**Only one replicate detected sufficient positive droplets for copy number calculation. The error reported here instead was the Poisson 95% confidence interval for 1 well as reported by the QuantaSoft software package.

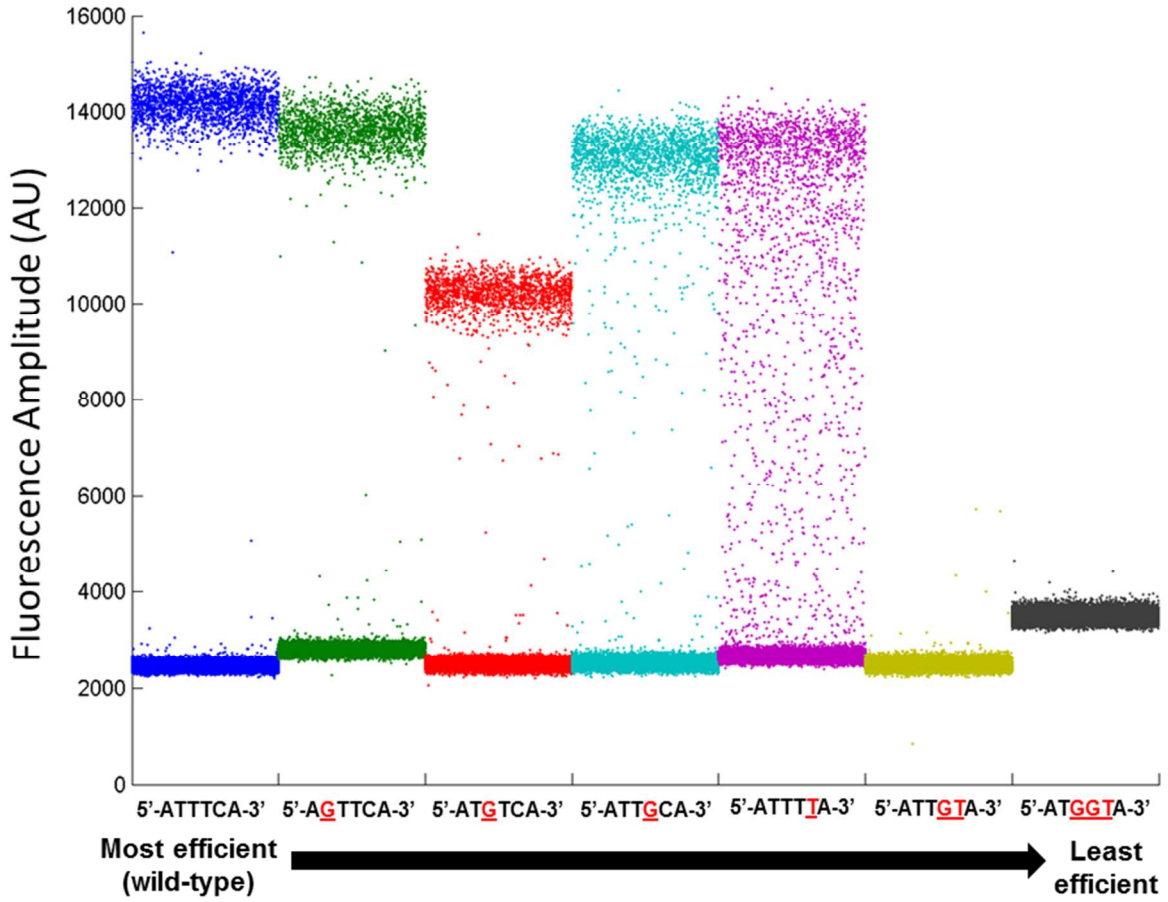


**Figure S-2. Effect of primer efficiency on droplet fluorescence amplitude.** Each colored column is one reaction (~20,000 droplets) with variations of the *BRAF* V600E primers without tails. All reactions contain 20ng of human diploid control template (NA18507), which is wild-type for the *BRAF* V600E mutation. The X-axis is labeled with the reverse primer sequence written in the 5' to 3' orientation with the mismatched base pair underlined in red. As the mismatched base pair moves towards the 3' end, the primer becomes less efficient and the end-point fluorescence amplitude decreases with more intermediate amplitude droplets.

**Table S-1. *FLT3* Copy number of colorectal patient samples.**

| Template | Source | EvaGreen Copy Number | StdDev (N=3) |
|---|---|---|---|
| 18507 | normal diploid DNA | 2.051 | 0.005 |
| 1668 | patient tumor | 3.089 | 0.025 |
| 1480 | patient tumor | 2.644 | 0.003 |
| 825 | patient tumor | 1.996 | 0.007 |
| 1563 | patient tumor | 1.530 | 0.007 |

**Note S-1. Statistical calculations for CNV and SNV analysis.**

Statistical significance calculations for Figures 2 and 3 were calculated by a one-tailed Student's t-test comparing experiments against the copy number measured in the null CNV and SNV cases (pure NA18507, which is diploid in *FLT3* and does not contain the *BRAF* V600E mutation). This method is similar to a previously described approach, where the log-ratio of the measured CNV or SNV is approximately normally distributed.[1] All calculated *P*-values in Figures 2 and 3 were smaller than a threshold of 0.05.

## REFERENCES

(1) Whale, A. S.; Huggett, J. F.; Cowen, S.; Speirs, V.; Shaw, J.; Ellison, S.; Foy, C. A.; Scott, D. J. *Nucleic acids research* **2012**, *40*, e82.